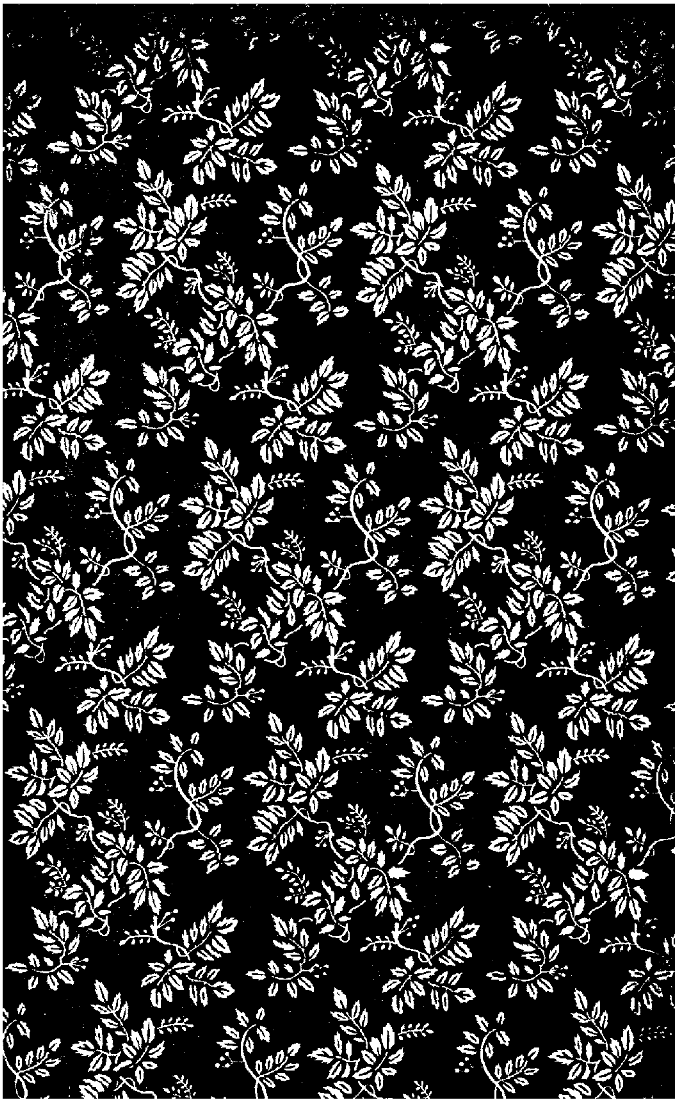

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EFFECT OF RÖNTGEN RAYS ON THE TOBACCO, OR CIGARETTE, BEETLE AND THE RESULTS OF EXPERIMENTS WITH A NEW FORM OF RÖNTGEN TUBE

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INTRODUCTION

The Röntgen tube used in experiments on the effect of Röntgen rays on the tobacco, or cigarette, beetle (*Lasioderma serricorne* Fabricius) described in this paper is a new form designed by Coolidge.[1] By this type of tube a much more powerful Röntgen-ray radiation can be maintained than was possible with the apparatus used in experiments of a similar nature previously made by the writer. The intensity and the penetrating power of the Röntgen rays produced are both under the complete control of the operator, and many of the factors limiting the use of other types of tubes for the special purpose desired are absent. The tube can be operated continuously for long periods without showing an appreciable change in either the intensity or the penetrating power of its resulting radiation. The starting and running voltage are the same. The resulting radiation is therefore homogeneous and of any desired penetrating power.

The ordinary forms of tubes used in previous experiments were incapable of being operated continuously without change in penetrating power. Owing to the fluctuation in intensity and penetrating power incidental to frequent adjustment, it was impossible to tell with any degree of accuracy the dosage and amount of radiation.

In previous experimental work with Röntgen rays it had been found that in sterilizing cigars or tobacco, small dosages are ineffective, from a practical standpoint. To be effective, the radiation must be intense, and it is evident that if the process can be successfully applied to commercial work, the apparatus used must be capable of producing and maintaining such radiation during the entire period required for the material treated to pass through the exposure chamber of the machine.

[1] Coolidge, W. D. A powerful Röntgen ray tube with a pure electron discharge. *In* Phys. Rev., s. 2, v. 2, no. 6, p. 409-430, 6 fig. 1913.

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TABLE I.—*Effect of Röntgen rays on development of the tobacco beetle*

[Experiments made at Schenectady, N. Y.[1]]

Experiment No.	Date.	Stage of insect.	Number tested.	Age.	Current (milliampere minutes).	Time.	Exposure (milliampere minutes).	Date examined.	Results.	Date examined.	Results.
				Days.		*Minutes.*					
1	Apr. 10	Egg	27	1	10	15	150	Apr. 16	None hatched	May 1	None hatched.
2	Apr. 12	do	95	1-2	15	10	150	Apr. 20	do	do	Do.
3	Apr. 13	do	35	1	15	10	150	May 1	do	Sept. 11	Tobacco uninjured and uninfested. Check heavily infested.
4	Apr. 14	do	50	1	15	10	150	do	do	do	Do.
5	Apr. 15	do	50	1	15	10	150	do	do	do	Do.
6	do	do	50	3	15	10	150	do	do	do	Do.
7	Apr. 16	do	50	2	15	10	150	do	do	do	Do.
8	do	do	110	1	15	10	150	do	do	do	Do.
9	do	do	50	2	15	10	150	do	do	do	Do.
10	do	do	50	2	15	10	150	do	do	do	Do.
11	Apr. 17	do	50	3	15	10	150	do	1 eggshell and 1 live larva	do	1 dead larva partly grown. Check infested. Larvæ, pupæ, and adults found.
12	do	do	50	3	15	10	150	do	None hatched	do	No signs of development. Tobacco uninjured. Check infested.
13	do	do	42	4	15	40	600	do	do	do	Do.
14	do	do	52	5	15	40	600	do	38 eggs hatched; 14 sterile	do	Several small dead larvæ found; no pupæ or adults; no evidence of any of the larvæ having developed.
15	do	do	52	1	15	10	150	do	None hatched	do	Tobacco uninfested and uninjured. Check box infested.
16	June 7	do	203	1-2	15	10	150	June 24 July 11	do do	Sept. 1 Dec. 12	Do. No evidence of any of the eggs having hatched.
17	do	do	20	1-3	15	10	150	June 24 July 11	do do	Sept. 1 Dec. 12	Do. Do.
18	do	do	82	4-5	15	10	150	June 24	2 dead larvæ in first instar; a few egg-shells; no live larvæ.	Sept. 1 Dec. 12	No signs of further development. Tobacco uninfested.
19	Apr. 17	Larval	21	([2])	15	40	600	May 3 May 28 July 11 Sept. 1	All alive 14 alive; 7 dead 2 alive; rest dead All dead; none reached adult stage; all check larvæ transformed by July 11.		

20	June 7	...do.....	35	(3)	15	40	600	June 24	21 alive; part seem inactive..........	Sept. 1 Oct. 1 Nov. 1	9 alive... 5 alive... 4 alive...	No signs of feeding observed. None of the larvæ reached the pupal stage. Treatment apparently stopped all development and activity; were somewhat shrunken and of an abnormal whitish color.
								July 11	12 alive, but in a dormant condition; none have transformed to pupal or adult stage; checks have all transformed to adults.	Dec. 11 Jan. 1, 1916. Jan. 10, 1916.	2 alive... 2 alive... All dead	
21	...do.....	Pupal...	20	....	15	40	600	June 16 July 11	8 alive; none transformed............ 4 live adults found...................	Aug. 1	Adults dead. No eggs laid.	
22	Apr. 17	Adult...	67	....	15	40	600	Apr. 17	Alive and active directly after exposure.	Oct. 1	Tobacco from jar containing exposed beetles and eggs examined; no signs of infestation.	
								Apr. 22	Large number of eggs deposited. 208 eggs kept under daily observation; rest of eggs placed with chewing tobacco in sealed jar.			
								May 30	None of the eggs hatched; eggs from check beetles hatched normally.			
23	June 7	...do.....	50	....	15	40	600	June 24	26 alive, 24 dead; large number of eggs found; none hatched; check beetles, 32 alive, 18 dead; eggs from check beetles hatched normally.	July 24	All adults in both exposed and check lot dead; none of the eggs from exposed beetles hatched.	
								July 11	Exposed lot, 7 alive; check, 5 alive; 126 eggs kept from exposed lot examined; none hatched.	Sept. 1	No signs of infestation in tobacco kept in jar which contained exposed adults. Tobacco in check jar infested.	

[1] The following were constant in all the experiments: Material exposed 7.5 inches from focal spot of tube; spark gap 5.5 inches, giving, at a humidity of 57°, a voltage of about 65,000.

[2] Partly grown.

[3] Partly grown; some nearly mature.

EXPERIMENTAL WORK

Eggs for the experiments were obtained by placing large numbers of tobacco beetles in jars containing leaf tobacco which had been sterilized by heat. The eggs were then placed between slabs of chewing tobacco in wooden boxes. The covers of the boxes were tightly sealed with adhesive tape. Control boxes containing approximately the same number of eggs as the treated boxes were prepared in a similar manner.

Infested tobacco containing larvæ, pupæ, and adults was also exposed in sealed wooden boxes. After exposure the insects were transferred to wooden boxes containing granulated tobacco which had been sterilized by heat. A corresponding number of specimens were kept as controls.

Exposure to the rays was made by placing the containers directly under the Röntgen tube at a distance of 7.5 inches from its focal spot. In order to guard against any effect of heat, a fan was kept blowing on the container while the exposure was made. The maximum temperature registered by a thermometer placed in the chamber was 91° F.

In the series of experiments tabulated 150 milliampere minutes (current of 15 milliamperes for 10 minutes or a current of 10 milliamperes for 15 minutes), with a voltage of 65,000, was the minimum dosage applied.

The material used in the experiments was kept under observation until January 10, 1916. Table I gives the details of the experiments. The notes included show the condition at different times. During the colder months the material was kept in an automatically regulated electric incubator in which suitable breeding conditions were maintained. The temperature was kept at 86° F. and the humidity at 80.

Eggs from exposed beetles were kept under daily observation. Part were kept in cells on microscope slides and part were kept on the leaf tobacco on which they were laid and placed between slabs of chewing tobacco. Most of the eggs which failed to hatch became shrunken and changed in color in about 10 days. Part remained plump and apparently normal for a considerable time. In eggs which were over 2 days old and in which embryonic development was well advanced when treated the partly developed larvæ could be seen within by examination with a microscope.

As will be seen in Table I (experiments 11, 14, and 18), hatching took place in some of the eggs which were over 3 days old. In experiment 14, which was made with eggs nearly hatched when treated, part of the eggs hatched, even though the dosage of 150 milliampere minutes, which was effective with the newly laid eggs, had been increased to 600 milliampere minutes.

Results of previous experiments, as well as those tabulated, indicate that in treatment of the egg stage heavier dosages are required to sterilize eggs which are nearing the end of the incubation period than are required to sterilize eggs newly laid.

In these experiments the larvæ hatched from treated eggs failed to develop. In several other series of experiments with Röntgen rays made by the writer and also in experiments made by Morgan and the writer,[1] eggs given lighter dosage hatched and development seemed normal, several generations of tobacco beetles being reared from some of the tobacco and cigars which contained treated eggs.

In the two experiments with larvæ (No. 19 and 20), no immediate effect as the result of exposure to the rays was noted. After a time the larvæ became inactive, somewhat shrunken, and changed in color, and no evidence of feeding could be observed. Nearly all remained in an inactive or dormant condition for long periods before death. Two larvæ exposed on June 7 (experiment 20) remained alive until January 10, 1916. All check larvæ used in this experiment had transformed to the adult stage by July 11. All treated larvæ died before reaching the pupal stage. With conditions under which the material used in the experiments was kept, the normal larval period of the tobácco beetle is about 40 days. All larvæ used in the experiments were partly grown when the experiment was made. No further growth could be noticed. In general, the effect of the heavy exposure given (600 milliampere minutes, voltage 65,000, distance from focal spot of Röntgen tube 7.5 inches) seems to have been to stop development and activity and to produce an inactive or dormant condition, and greatly to prolong the larval period.

The results of all previous experiments with larvæ given comparatively light exposures had shown entirely negative results.

In the experiment with pupæ (experiment 21) the number of pupæ used was not sufficiently large to permit the drawing of positive conclusions. Of the 20 specimens treated, only 4 reached the adult stage. These seemed normal, but died without laying eggs.

In the two experiments with adults (experiments 22 and 23), the results obtained were very similar. The exposure given apparently did not affect the length of life or the activity. Mating was observed and large numbers of eggs were laid. None of the eggs from the exposed beetles hatched, while eggs from the check beetles hatched normally.

Egg clusters of the tent caterpillar (*Malacosoma americana* Fabricius) and the white-marked tussock moth (*Notolophus leucostigma* Smith and Abbot) were used. With both of these species the period of incubation is very long, eggs deposited in summer or fall not hatching until the following season. An exposure of 150 milliampere minutes was given. Other conditions of the experiment were the same as in experiment 7 made with eggs of the tobacco beetle, details of which are given in Table I. The experiment was made on April 16. The egg clusters treated contained something over 1,000 eggs of each species. The same number of clusters were kept as checks. Both experiments gave nega-

[1] Morgan, A. C., and Runner, G. A. Some experiments with Röntgen rays upon the cigarette beetle Lasioderma serricorne Fabr. *In* Jour. Econ. Ent., v. 6, no. 2, p. 226–230. 1913.

tive results, hatching being apparently normal in treated eggs of both species.

The eggs of both the tent caterpillar and the tussock moth were nearing the end of the incubation period when treated. In eggs of the tent caterpillar embryonic development is practically completed in the fall, the larvæ remaining in the eggshells over the winter and emerging on the appearance of warm weather in the spring.

SUMMARY

Under laboratory conditions tests made with a Röntgen-ray tube permitting a high-energy input and giving an intense and powerful radiation gave results which promise that the X-ray process may be successfully used in treatment of cigars or tobacco infested with the tobacco, or cigarette, beetle.

Heavy dosages must be given, as is indicated by the exposure given in the series of experiments tabulated in this paper.

In treatment of the egg stage, heavier exposures are required to sterilize eggs which are near the hatching point than are required to sterilize eggs newly laid.

In experiments performed by the writer a dosage equivalent to 150 milliampere minutes exposure with a spark gap of 5.5 inches gave satisfactory results with eggs in tobacco placed 7.5 inches from the focal spot of the tube. With this exposure the eggs in which embryonic development was well advanced hatched, but in all cases where these larvæ were kept under observation they failed to reach the adult stage.

The minimum lethal dosage at a given distance from the focal spot of the Röntgen tube used has not been determined.

In two separate experiments adults were given an exposure of 600 milliampere minutes (amperage × time), with a spark gap of 5.5 inches, giving an approximate voltage of 65,000, with humidity at 57. The distance from the focal spot of the Röntgen tube was 7.5 inches. The results are as follows:

(1) No effect on length of life was apparent, as the beetles died at about the same rate as the same number of beetles kept as a check.

(2) Large numbers of eggs were deposited after exposure. These eggs were infertile. Eggs laid by the check beetles hatched normally.

Larvæ were given an exposure of 600 milliampere minutes, other conditions of the experiment being the same as in the experiments with adults given above. While no immediate effect was apparent, the treatment had the effect of stopping activity and development, the larvæ remaining in a dormant condition for a prolonged period. All treated larvæ died before reaching the pupal stage.

STIMULATING INFLUENCE OF ARSENIC UPON THE NITROGEN-FIXING ORGANISMS OF THE SOIL

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INTRODUCTION

Arsenic, when applied to a soil, has been found to stimulate the ammonifying (Greaves, 1913c)[1] and especially the nitrifying organisms of that soil. The stimulation varied greatly with the form, quantity, and method of applying the arsenic. Furthermore it was found that very large quantities of arsenic had to be applied to a soil before its toxic effect became marked. This toxic effect became pronounced only when quantities of arsenic which far exceeded those found in any of the cultivated soils (Greaves, 1913b) had been applied. Therefore it was desirable to determine its influence and mode of action upon the nitrogen-fixing powers of the soil. For, even though arsenic does not inhibit the action of the ammonifiers or nitrifiers, if it stops or materially retards the nitrogen-fixing organism, it can not be said that arsenic is not injurious to the soil flora. To determine this point the following study has been made.

EXPERIMENTAL WORK

The soil used in the first part of this work was the same as that used by the author in the previous series. It is a typical bench soil, a sandy loam fairly high in calcium and iron content and supplied with an abundance of all the essential elements of plant food with the exception of nitrogen, which was low, a characteristic of arid soils.

The determination of the nitrogen-fixing powers of the soil was made as follows: Tumblers covered with Petri dishes were sterilized, and into these were weighed 100-gm. portions of the air-dried soil and 2 gm. of mannite, which were then carefully mixed. Sodium arsenate was added from a standard solution with the proper proportion of sterile distilled water and the mixture thoroughly stirred with a sterile spatula. The other arsenical compounds were added in the dry state and then carefully mixed. Sufficient sterile distilled water was added to make the moisture content of the soil 18 per cent. The tumblers and contents were weighed and the moisture content made up weekly to the initial concentration.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 414-416.

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The samples were incubated at 28° to 30° C. for 18 days and the total nitrogen determined. The tumblers and contents at the end of this time were placed in an electric incubator and kept at 95° C. until dry. The soil was then ground in a mortar, after which 20-gm. portions were weighed and placed in Kjeldahl flasks. The nitrogen was then determined according to the Lipman and Sharp (1912) method. The determinations were all made in duplicate and compared with sterile blanks, so that each result reported is the average of two or more closely agreeing determinations. The compounds used were sodium arsenate, lead arsenate, cupric aceto-arsenite (Paris green), arsenic trisulphid, and zinc arsenite. In each case the quantity of the compound added was such as to give equivalent quantities of arsenic. The results reported as milligrams of nitrogen per 100 gm. of soil are given in Table I.

TABLE I.—*Quantity of nitrogen (milligrams) fixed in 100 gm. of soil during 18 days with varying amounts and different forms of arsenic*

Arsenic.	Sodium arsenate.	Lead arsenate.	Paris green.	Arsenic trisulphid.	Zinc arsenite.
P. p. m.					
0	18.2	16.1	15.22	9.8	9.1
20	22.4	16.0	13.72	11.2	11.9
40	14.0	16.4	13.02	14.0	9.7
80	14.0	18.9	14.00	15.4	9.6
120	15.0	21.0	8.82	16.2	10.5
160	14.4	21.0	8.32	16.4	9.7
200	14.0	21.7	7.42	14.0	8.4
240	12.6	16.8	6.72	12.8	8.4
280	0	16.1	6.02	11.2	8.4
320	0	16.0	6.00	11.2	9.0
360	0	16.6	6.02	9.8	9.1
400	0	16.8	5.22	9.8	9.1
0	18.2	16.1	15.22	9.8	9.1

In this series the concentration of the arsenic was not carried above 400 p. p. m., for previous work had shown that the main stimulation occurs below this concentration. Furthermore the arsenic occurring in agricultural soils seldom exceeds 150 p. p. m., so it is likely that in agricultural soils it will never be found to exceed the quantity used in this work.

The results reported in the above table bring out some very interesting facts and show that the nitrogen-fixing organisms are very similar to the nitrifying organisms in so far as their relations to arsenic are concerned. The addition of 20 p. p. m. of sodium arsenate stimulates their action and 40 p. p. m. or more have a toxic influence. When the concentration of arsenic reaches 280 p. p. m., it stops all nitrogen-fixing activity. The toxic influence which becomes so very prominent above this concentration must be due entirely to the arsenic and not to the sodium ion, as Lipman and Sharp (1912) have added many times this

quantity of sodium in the form of sulphates, chlorids, and carbonates to the soil without retarding its nitrogen-fixing power.

The lead arsenate at the lower concentrations has no influence upon the nitrogen-fixing powers of the soil, but when the concentration reaches 80 p. p. m. a stimulating influence becomes quite perceptible. This continues until the concentration exceeds 200 p. p. m. Above this concentration the nitrogen fixed, within experimental error, is the same as that fixed in the untreated soil. It is interesting to note that the compound does not become toxic, even when the quantity added reaches 400 parts of arsenic per million parts of soil. This series shows a very close similarity to the nitrification series previously reported, and it is quite likely that part of the stimulating influence is due to the lead ion.

Paris green is toxic even in the lowest concentration used, and the toxicity increases as the quantity of Paris green added increases. This toxicity is due mainly to the copper ion. However, as was shown in the ammonification and nitrification work, the quantity of soluble arsenic present would be much higher where the Paris green was added than where the other compounds were used. The fact that no stimulation occurs in the Paris-green series points to the conclusion that the toxicity of the copper must increase much more rapidly than the stimulating influence of the arsenic. Yet it is quite possible that if a lower concentration of the substance had been taken a stimulation would have been noted.

Arsenic trisulphid stimulates in the lowest concentration tested and increases in stimulating influence until a concentration of 160 p. p. m. is reached. In concentrations above this its stimulating influence decreases. In concentration above 320 p. p. m. there is fixed no more nitrogen in the presence than in the absence of arsenic. But even at the highest concentration tested (400 p. p. m.) this compound exerts no tonic influence on the nitrogen fixers.

Zinc arsenite probably stimulates slightly in low concentrations, but aside from this it has little apparent influence on the nitrogen-gathering organisms. Had fresh soil been used in this series, greater stimulation would have been noted, as was found by later work.

The amount of nitrogen fixed in the untreated soil of the above series shows a marked variation. This is probably due to various factors, chief among which is the fact that the nitrogen-fixing powers of the soil with sodium arsenate, lead arsenate, and Paris green were made in the order named on the air-dried soil soon after it had been brought to the laboratory. In the case of the arsenic trisulphid and zinc arsenite the soil had been in the laboratory in an air-dried condition for about two months before the determinations were made, but each set of samples within each series was handled in exactly the same manner, and the samples are directly comparable within each set, as has been the case in the previous

discussion. In order to make those containing different forms of arsenic more nearly comparable with each other—that is, the lead arsenate with the arsenic trisulphid, etc.—the nitrogen fixed in the untreated soil has been taken as 100, and from this the ratio has been calculated with each of the concentrations of arsenic. This gives us more nearly comparable results, which are shown in figure 1.

Comparing these results with those obtained for the ammonification and nitrification series (Greaves, 1913c), we find a marked similarity existing between them. In all of the series there is a marked stimulation with all of the compounds except Paris green. The arsenic trisulphid

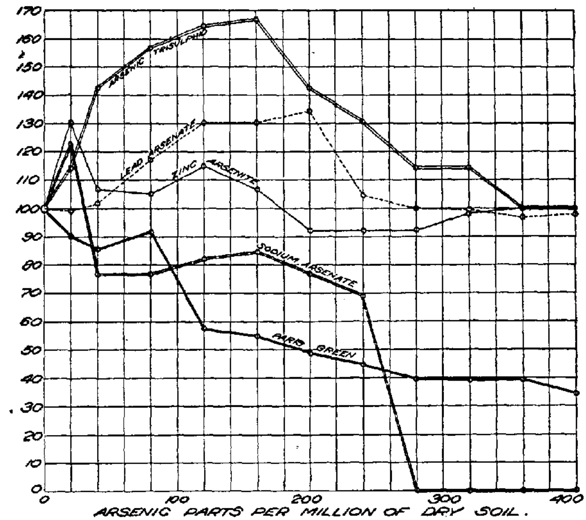

FIG. 1.—Graph showing the action of five compounds of arsenic on nitrogen fixation in dry soil. (Nitrogen fixed in untreated soil equals 100.)

stimulates growth much more in the nitrogen-fixing series than it does in the other series. The arsenic trisulphid has the greatest stimulating influence, followed in the order named by lead arsenate, zinc arsenite, and sodium arsenate. Paris green was the only compound tested which exerted no stimulating influence. It may be seen that the maximum stimulation was not obtained when equivalent quantities of arsenic in the various forms are applied to the soil. Hence, it seems possible that a relationship may exist among the various cases in the water-soluble arsenic found. In order to answer this, determinations were made of the water-soluble arsenic existing in the soil. The soil and arsenic,

together with 2 gm. of mannite, were placed in sterile tumblers, the water content made up to 18 per cent, and then incubated at 28° C. for 18 days. At the end of this period the soil was transferred by means of 1,000 c. c. of carbon-dioxid-free distilled water to large acid bottles. The mixture was left in these bottles, with occasional shaking, for 8 days, then filtered and the arsenic determined in an aliquot part (Greaves, 1913d). In another set the various forms of arsenic were mixed with 100-gm. portions of soil and 2 gm. of mannite and the water-soluble arsenic determined as above without incubation.

The results are given in Table II as milligrams of water-soluble arsenic occurring in 100 gm. of the soil both before and after the three weeks' incubation. Each reported result is the average of three or more closely agreeing determinations.

TABLE II.—*Quantity of water-soluble arsenic (in milligrams) in 100 gm. of soil before and after three weeks' incubation*

Treatment.	Lead arsenate.	Arsenic trisulphid.	Sodium arsenate.
Arsenic added	16. 00	16. 00	2. 00
Arsenic found before incubation	1. 04	. 14	1. 08
Arsenic found after incubation	1. 26	1. 42	1. 44
Average	1. 15	. 78	1. 26

The arsenic in each case became more soluble as bacterial activity progressed. This is especially marked in the soil containing arsenic trisulphid, which yielded 10 times the water-soluble arsenic after incubation that it did before. A remarkably close agreement is found to exist among the results obtained for water-soluble arsenic at the close of the incubation period, which shows that the maximum stimulating influence is obtained when soil contains between 10 and 15 p. p. m. of water-soluble arsenic. This is a quantity that exceeds that found in agricultural soil (Greaves, 1913b); hence, the influence of the arsenic occurring in soil must be to increase and not to retard nitrogen fixation. The maximum fixation varies with the form of arsenic applied. This is undoubtedly due, as will be pointed out later, to the elements accompanying the arsenic, which may have either a retarding or an accelerating influence upon the bacterial activity.

The finding of this marked stimulating influence of arsenic upon the nitrogen-fixing powers of soil raises a number of very interesting and important questions. Some of these are: (1) Does this stimulating influence exist in other soil or is there something inherent within this particular soil which makes its bacterial flora susceptible to the influence of arsenic? (2) Is the stimulating influence brought about by the retarding of injurious species or is it a direct stimulant to the soil organisms?

(3) Do the arsenic and arsenic compounds act as a source of energy to the nitrogen-fixing organisms or do they so influence the soil flora that it can utilize more economically the carbon compounds available? (4) What nitrogen-fixing organisms are there in the soil which are influenced by arsenic?

In order to find whether arsenic influences the nitrogen-fixing powers of other soils in a similar manner, three other soils were tested with and without arsenic. The soils vary greatly in chemical and physical composition. Soil A is a black loam of very light texture and, for an arid soil, high in nitrogen and humus. It is well supplied with phosphorus, potassium, and calcium carbonate and grew potatoes for 23 years. After this it was planted to oats for 2 years, and during the past 4 years has been planted in alfalfa. It has received some manure. Soil B is a sandy loam of much lighter color than soil A and contained much less humus and nitrogen, but an abundance of other elements. It has been cultivated for 28 years and during this time has been fallowed two summers. The remainder of the time it has been planted in wheat. Soil C is a heavy clay almost devoid of humus. The nitrogen is low, but the soil is well supplied with phosphorus, potassium, and calcium carbonate. While wet it is exceedingly sticky, and on drying it bakes like adobe. It has been tilled for 23 years, and during this time it has been fallowed for 3 years. The remainder of the time it has been in wheat. While it has received no manure during this time, it is still very productive. All of the soils are very fertile and well supplied with Azotobacter, and previous work has shown them to have high nitrogen-fixing powers.

The soils were all air-dried in the dark for 24 hours, ground in a mortar, sieved, weighed, and placed in sterile tumblers. Some were mixed with mannite and arsenic, others with mannite, while still others received only arsenic. They were all incubated in the regular manner, and the nitrogen determined as in the previous series. The results are given in Table III. Each reported result is the average of six closely agreeing determinations.

A marked stimulation is found in every case where the arsenic and mannite were applied to the soil, as compared with the results obtained where the mannite only was applied. The action of the various arsenical compounds follows the same order in each of these soils that it did in the first soil tested, being greatest with the lead arsenate and least with the sodium arsenate. The nitrogen fixed in the presence of arsenic but in the absence of mannite is usually considerably higher than that fixed in the presence of mannite and absence of arsenic. It would not be right to conclude from these results that the arsenic compounds furnish a source of energy to the nitrogen-fixing organisms, for these soils (Greaves, 1914; p. 456) have been found to fix appreciable quantities of nitrogen when incubated with an optimum moisture content without the addition of any carbon compound. It is likely that the arsenic makes the nitrogen-

gathering organism use more economically its usual source of carbon, which in the absence of mannite is probably the plant débris which has been slowly added to the soil. The belief that this is the case is strengthened by the fact that soil rich in organic matter (soil A) acts practically the same in the absence of mannite and presence of arsenic as it does when both arsenic and mannite are added to the soil. The clay soil (C), which is low in organic matter, acts about the same in the absence of arsenic as in the absence of mannite. It is interesting to note that in soils B and C the total fixation in the soil containing mannite plus that fixed by the soil containing arsenic approximates the total fixation in the series in which both arsenic and mannite are present.

TABLE III.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil with and out arsenic*

Soil.	16 mgm. of arsenic, 2 gm. of mannite, added to soil.	16 mgm. of arsenic, no mannite, added to soil.	No arsenic, 2 gm. of mannite, added to soil.	Total of columns 2 and 3.
LEAD ARSENATE				
A	17.0	16.8	6.7	24.5
B	16.8	9.8	4.0	13.8
C	10.5	5.3	6.3	11.6
Average	14.7	10.6	6.0	16.6
ARSENIC TRISULPHID				
A	16.3	15.6	13.8	29.4
B	12.6	7.0	7.6	14.6
C	10.6	5.6	4.2	9.8
Average	13.1	9.4	8.5	17.9
SODIUM ARSENATE				
A	7.8	6.3	6.3	12.60
B	7.0	4.9	3.3	8.20
C	9.2	8.4	7.0	15.40
Average	8.0	6.5	5.5	12.0

In all of the tests so far reported the incubation period has been 18 days. Longer periods of incubation may give results very different from those so far obtained, for the stimulating influence of arsenic may be of short duration, and we may find later a slowing up of the reaction, or, inasmuch as we are dealing with the algebraic sum of many reactions which are taking place in the soil, we may find it to be negative. An attempt was made to determine this by the following experiment: 100-gm. portions of the high-humus soil (A) were mixed with 0.0728

gm. of lead arsenate and the moisture content made up to 18 per cent and then weighed. One-half of the samples thus prepared were sterilized in the autoclave and all of them placed in an incubator at a temperature of from 28° to 30° C. The moisture was made up weekly to its initial content. Beginning at the end of 20 days, six samples, three autoclaved and three not autoclaved, were used for the making of duplicate total-nitrogen determinations. The average excess of nitrogen in the unsterilized soil over that in the sterilized is given in Table IV.

TABLE IV.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil containing 0.0728 gm. of lead arsenate*

Time incubated.	Nitrogen.	Time incubated.	Nitrogen.
Days.	*Mgm.*	*Days.*	*Mgm.*
20	12.32	96	1.00
30	−12.40	162	3.80
44	−16.40	172	6.20
66	−8.20		

The greatest quantity of nitrogen was obtained at the end of 20 days. During the next 10 days, however, 24.72 mgm. of combined nitrogen disappeared. During the next 14 days there was a loss of only 4 mgm. From this time on there was a gradual increase in the amount of combined nitrogen found within the soil up to the end of the experiment, but even after 172 days' incubation there was less nitrogen in the soil than there was at the end of 20 days.

The great loss of nitrogen can not be entirely charged up to the arsenic added, for other workers (Ashby, 1907; Hoffmann and Hammer, 1910, p. 164) have noted, when working with impure cultures, a loss of nitrogen on prolonged incubation in the absence of arsenic. The loss is probably due to the soil's becoming compact, with the production of anaerobic conditions. This, assisted by the protozoa (Miller, 1914, p. 217), which appropriate too large a share of the limited supply of oxygen in the soil, prevents entirely the activity of the aerobic nitrogen-fixing organisms and greatly stimulates the activity of the denitrifying organisms of the soil. This can, however, only partly account for the phenomena; otherwise there would be a continual decrease in the nitrogen as the soil became more compact.

The fact that aeration plays a considerable part in the reaction is brought out by the following experiment, which differs from the preceding only in that the soil was thoroughly stirred, thus aerating it each time before making up the moisture content. The results of this experiment are given in Table V.

TABLE V.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of aerated soil with and without the addition of arsenic after different periods of incubation*

Days incubated.	Nitrogen fixed in soil containing 0.0728 gm. of lead arsenate.	Nitrogen fixed in untreated soil.	Days incubated.	Nitrogen fixed in soil containing 0.0728 gm. of lead arsenate.	Nitrogen fixed in untreated soil.
20	8.12	2.58	66	9.38	14.00
30	5.88	3.92	96	4.90	2.52
44	8.26	3.78	162	2.80	4.20

These results show conclusively that it was the lack of air in the former series which caused such great losses of nitrogen and that they could in no way be attributed to the arsenic added. This series was stirred but once a week and after the stirring the moisture content was made up to the optimum so that the soil became quite compact. It is quite likely that greater care in the aeration of the soil would have reduced very materially the loss of nitrogen which was observed in this series. In the first stages of the experiment the soil containing arsenic gained the greater quantity of nitrogen, while in the later stages the soils containing no arsenic were the highest. If, however, an average of the quantity found in each soil is taken, it will be found to be considerably higher in the soil containing arsenic than in the other.

It was thought that some of the questions referred to in the first part of this article could be answered more readily with the solution method than with soil. For this reason a series was incubated using a solution of the following composition:

Dibasic potassium phosphate (K_2HPO_4)	0.2 gm.
Magnesium sulphate ($MgSO_4$)	.2 gm.
Calcium chlorid ($CaCl_2$)	.02 gm.
Ferric chlorid (Fe_2Cl_6)	1 drop (10 per cent solution).

This was made up to 1,000 c. c. with tap water and distributed in 100 c. c. portions into 750 c. c. Erlenmeyer flasks. One gm. of calcium carbonate was added to each, and the flasks were then sterilized and inoculated. One series was inoculated with *Azotobacter vinelandii*. This was done by making a suspension in sterile tap water of the organism and adding 5 c. c. of this suspension to each flask. In the other series the inoculating medium was 10 gm. of soil. The solutions were incubated at 28° to 30° C. for 18 days, and then the nitrogen determined in the manner previously outlined. The results are given in Table VI and are reported as milligrams of nitrogen fixed in 100 c. c. of the solution. Each reported result is the average of three closely agreeing determinations.

TABLE VI.—*Quantity of nitrogen (in milligrams) fixed in 100 c. c. of nutritive solution with and without the addition of arsenic*

Treatment.	Inoculated with *Azotobacter vinelandii*.	Soil + 0.0728 gm. of sterilized lead arsenate.	Soil + 0.0728 gm. of unsterilized lead arsenate.
Nutritive solution + 1.5 gm. of mannite	14.12	15.16	15.77
Nutritive solution + 1.5 gm. of mannite and 0.0728 gm. of lead arsenate	0	14.79	13.72
Nutritive solution + 0.0728 gm. of lead arsenate	0	1.45	.52
Nutritive solution + 1.5 gm. of mannite and 0.0272 gm. of arsenic trisulphid	.5	5.98	2.05
Nutritive solution + 0.0272 gm. of arsenic trisulphid	0	.28	.08

After the first series had been completed, it was thought possible that the heat in the autoclave had changed the solubility of the arsenical compounds and that this was the reason there was no fixation in the solution with arsenic. For this reason analyses were made of the soluble arsenic in 100 c. c. of the nutritive solution containing arsenic both before and after autoclaving. The determinations were made as previously outlined. The lead arsenate yielded 0.91 mgm. of soluble arsenic before autoclaving and 0.85 mgm. after autoclaving. The arsenic trisulphid yielded 0.40 mgm. before autoclaving and 0.42 mgm. after autoclaving.

The results indicate conclusively that the toxicity of the compound is not due to a difference in the solubility of the compound produced by the heat. In order to make sure of this, a series was arranged in which the arsenic was added just before inoculation and after the solution had been autoclaved. These results are given in the last column of Table VI and are slightly lower than those previously obtained with the arsenic. The *A. vinelandii* fixed no nitrogen in the presence of the arsenic. Even where the soil was used as the inoculating medium, the lead arsenate retarded nitrogen fixations to a certain extent. The toxic influence of the arsenic sulphid is very pronounced. These results show the care which must be used in drawing conclusions from the Remy-solution method as to what is to be expected in soils. They greatly strengthen the contention of Jönsson (1896) that the fact that Nobbe (1884) found arsenic solutions to be toxic to seedlings in water culture and concluded that arsenic, even in small quantities, is extremely toxic to plants does not indicate that these solutions will be toxic when in the soil. The results herein reported show arsenic to be extremely toxic to nitrogen-fixing organisms while in solution, but the same concentration in the soil is not only devoid of toxicity but acts as a powerful stimulant. This therefore establishes for the bacteria what Kanda (1904, p. 16) found to be true for the higher plants—namely, that dilute solutions of sub-

stances may be toxic when used in water culture, but that the same quantities when placed in the soil may act as stimulants.

The results reported for *A. vinelandii*, when considered in connection with those obtained for the soil, make very problematic the part played by Azotobacter, especially *A. vinelandii*, in these soils. The exact mode of action of the arsenic also remains a question. For these reasons the soil used in the first series was plated and the main nitrogen-fixing organisms isolated. Three types of Azotobacter were obtained. These have been designated Azotobacter A, Azotobacter B, and Azotobacter C. Azotobacter A has a nitrogen-fixing power of 6.86 mgm. of nitrogen per gram of mannite in Ashby solution, Azotobacter B a nitrogen-fixing power of 5.00 mgm., and Azotobacter C a nitrogen-fixing power of 6.44 mgm. of nitrogen.

The preceding results have shown that little information of value can be obtained by the solution method. Therefore another series was planned in which 100-gm. portions of the soil used in the first series were weighed into covered sterile tumblers and autoclaved at a temperature of 120° C. for 30 minutes, cooled, and the moisture content made up to 18 per cent. The soil was then inoculated with the various organisms which had been isolated. The soil portions were incubated for 18 days, the moisture content kept constant, and then the total nitrogen determined. Sterile blanks were incubated and analyzed as checks. Each reported result is the average of four or more closely agreeing determinations, so that the analytical error has been reduced to a minimum. The results are given in Table VII.

TABLE VII.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil with and without arsenic and inoculated with various nitrogen-fixing organisms*

Inoculating organism.	Milligrams of nitrogen fixed in 100 gm. of soil treated with—		
	2 gm. of mannite, 0.0728 gm. of lead arsenate.	2 gm. of mannite, no arsenic.	0.0728 gm. of lead arsenate, no mannite.
Azotobacter A	15.60	21.70	3.01
Azotobacter B	24.15	14.70	8.80
Azotobacter C	18.20	18.20	4.90
Azotobacter A and B	26.31	22.05	5.81
Azotobacter A, B, and C	18.40	17.70	6.65

The results reported above show for each organism a fixation much higher in the soil than was found in the solution. The results without arsenic, but with mannite, are as high as are reported in Table I with both mannite and arsenic combined, a fact which would seem to indicate that arsenic acts upon injurious species. This, however, does not account for the entire phenomenon, for we find in this series a very small fixation of nitrogen in the absence of mannite and presence of arsenic, while in

the ordinary soil with its mixed flora as great a fixation was obtained in the presence of arsenic as in the presence of only mannite. This probably indicates that some of the stimulation is due either to the fact that the arsenic acts upon allied species which are gathering carbon that can be used by the Azotobacter, or else to the fact that some species, possibly the cellulose ferments, are stimulated so that they render available to the Azotobacter the carbon-carrying compounds of the soil faster in the presence of arsenic than in its absence. Only one of the organisms isolated, Azotobacter B, is directly stimulated by arsenic. The stimulation, however, is very large in this case. It also fixes large quantities of nitrogen in the presence of arsenic and absence of mannite. These results are complicated by the carbonaceous material which occurs in the soil. For this reason a series similar to the above was incubated, using silica sand in place of the soil. The silica used was devoid of organic matter and had the following composition:

	Per cent.
Silicon dioxid (SiO_2)	97.5
Ferrous oxid (FeO)	.1
Aluminum oxid (Al_2O_3)	1.7
Calcium oxid (CaO)	.2

One-hundred gm. portions of this were sterilized in covered tumblers, and to each was added 1 gm. of calcium carbonate and 18 c. c. of sterile distilled water containing 0.02 gm. of potassium phosphate, 0.02 gm. of magnesium sulphate, and 0.002 gm. of calcium chlorid. The tumblers were inoculated with the various nitrogen-fixing organisms incubated with a constant moisture content at 28° C. for 18 days, and then the nitrogen determined as in the previous series. They were all compared with sterile blanks. The results are given in Table VIII as milligrams of nitrogen fixed in 100 gm. of sand. Each reported result is the average of six or more closely agreeing determinations.

TABLE VIII.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of quartz sand with and without arsenic*

Inoculating material.	Sand and Ash-by solution, +0.0728 gm. of lead arsenate.	Sand and Ash-by solution, no arsenic.	Sand and Ash-by solution, no mannite, +0.0728 gm. of lead arsenate.
10 c. c. of soil extract	19.60	10.50	4.70
Azotobacter A	17.01	22.61	0
Azotobacter B	13.84	12.60	0
Azotobacter C	15.10	16.80	0

Qualitatively, the above results are the same as those obtained with the soil. Azotobacter B was the only one of the three organisms stimulated by the arsenic. Where the mixed flora were used, the stimulation was very marked, but the fixation in the absence of arsenic where either Azotobacter A or Azotobacter C was used is about the same as that

obtained in the presence of arsenic where the soil extract was used. This fact would seem to indicate that the main stimulation brought about by arsenic is due to its action upon injurious species. The results obtained in the presence of arsenic and absence of mannite indicate that the Azotobacter can not use the arsenic as a source of energy. The small fixation where the soil extract was used may be due to the nitrogen-fixing organisms obtaining a small quantity of carbon compounds from algæ which may have grown in the complex flora.

The results given in Table VII pointed strongly to the conclusion that the stimulating influence of the arsenic was due in part to an indirect action upon the nitrogen-fixing organisms, possibly an action which it exerts upon the cellulose ferment. A series was therefore arranged in which the cellulose ferments were used in connection with the Azotobacter.

In this series 100-gm. portions of the high humus soil (A) were placed in covered tumblers and sterilized in the autoclave and then treated as in Table IX. The Azotobacter was inoculated into 100 c. c. of Ashby solution. After three days the solution was thoroughly shaken and 5 c. c. of the solution were added to the sterile soil. The cellulose ferment was added by making a suspension of the organism in sterile distilled water and adding 5 c. c. of this to the soil. The moisture content was made up to 18 per cent and incubated for 18 days. Six samples of each were used, so that the results reported are the averages of six closely agreeing determinations. The results are given in Table IX. The cellulose ferments used were *Bacillus rossicus*, isolated by Kellerman, McBeth, and others (1913) from Geneva (N. Y.) soils, and *Pseudomonas effusa*, isolated by the same investigators from the soils used in this work.

TABLE IX.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil with and without arsenic in the presence and absence of cellulose ferments*

Treatment.	Nitrogen gained.
Azotobacter chroococcum	14.70
Azotobacter chroococcum, 0.0728 gm. lead arsenate	14.28
Azotobacter chroococcum, *Bacillus rossicus*	26.18
Azotobacter chroococcum, *Bacillus rossicus*, 0.0728 gm. of lead arsenate	28.00
Azotobacter chroococcum, *Pseudomonas effusa*	13.30
Azotobacter chroococcum, *Pseudomonas effusa*, 0.0728 gm. of lead arsenate	22.68
Azotobacter B	14.46
Azotobacter B, 0.0728 gm. of lead arsenate	21.00
Azotobacter B, *Bacillus rossicus*	15.20
Azotobacter B, *Bacillus rossicus*, 0.0728 gm. of lead arsenate	19.60
Azotobacter B, *Pseudomonas effusa*	14.00
Azotobacter B, *Pseudomonas effusa*, 0.0728 gm. of lead arsenate	21.00

In this series, as in the previous series in which *A. chroococcum* was used, it did not fix as much nitrogen in the presence of arsenic as it did in the absence of it. *A. chroococcum* fixes nearly twice the quantity in the

presence of *B. rossicus* as in its absence, and when arsenic is added to the two there is an even greater fixation. This is also the case with *P. effusa;* measured in terms of the increased nitrogen fixed by *A. chroococcum*, it may therefore be safely concluded that both of the cellulose ferments are stimulated by lead arsenate.

The Azotobacter B differs from the *A. chroococcum* in that it is directly stimulated by the arsenic, but is not as greatly helped by the cellulose ferment. In this case the lead arsenate greatly stimulates the activity of the cellulose ferments, and the stimulating influence is much greater with *P. effusa*, the normal habitat of which is this soil, than it is with *B. rossicus*. Hence, from this work it is safe to conclude that the cellulose organisms, so far as arsenic is concerned, obey the same laws as do the ammonifying, nitrifying, and nitrogen-fixing organisms of the soil.

It has been noted throughout all of this work that the soil taken direct from the field was stimulated to a much greater extent by the arsenical compounds than was the air-dried soil. Furthermore, it was noted that the soil which had stood in the laboratory for a great length of time was stimulated only very slightly by arsenic. For these reasons a series of experiments was planned to throw more light upon this substance or organism which disappears on drying.

Fred (1911) has suggested the use of filter paper for the separation of the protozoa. Later this has been shown by Kopeloff and others (1915) to be quite effective. Using this suggestion, 100-gm. portions of soil were placed in tumblers. To half of them was added 0.0728 gm. of lead arsenate, and the mixture was autoclaved until free from bacterial life. They were all inoculated with 10 c. c. of a solution obtained by shaking 100 gm. of soil in 1,000 c. c. of sterile water and then filtering through three thicknesses of a fine grade of quantitative filter paper, after which they were incubated and nitrogen determined as in the previous set. The results are given in Table X as milligrams of nitrogen per 100 gm. of soil. All results are averages of six determinations made on that number of incubated samples.

TABLE X.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of sterile soil inoculated with filtered soil extract, with and without arsenic*

Time incubated.	0.0728 gm. of lead arsenate.	No lead arsenate.
Days.		
20	16.10	14.70
30	3.08	2.52
44	2.80	.30
66	1.94	.14
96	.28	.28
162	.78	.85

It probably would have been better if in every case untreated soil could have been incubated with the variously treated soil, but this so greatly increased the number of determinations that it was not thought advisable. Furthermore, all the work has been done on the high-humus soil, A, without the addition of any carbohydrate, and repeated determinations have shown that the arsenic more than doubles the nitrogen fixed in the soil in 20 days, so that the absence of the stimulation can be safely attributed to the treatment. In the above results, it is readily seen that the soil extract on passing through filter paper loses to a very great extent its power of being stimulated by arsenic. Hence, it is safe to conclude that the main stimulating influence of arsenic upon nitrogen fixation is due to its suppressing something which is found in the soil and which is removed by the filter paper.

That this factor is to a great extent the same as is removed by heat is shown by the results reported in Table XI. The arrangement of this series of experiments was as follows: 100-gm. portions of the soil were weighed into covered tumblers. To one-half of the set was added arsenic—0.0728 gm. to each 100 gm. of soil. The tumblers were all carefully sterilized and half of them were placed in the incubator in the sterile condition. To the others was added a soil extract prepared by shaking one part of soil with two parts of sterile distilled water for three minutes. After standing for about five minutes the liquid was decanted and 10 c. c. portions of this were used to inoculate the soil. Before inoculating, this extract was placed in thin-walled test tubes in 10 c. c. portions and then held at the required temperature for exactly 15 minutes before adding to the soil. The moisture content was made up to 18 per cent and the whole incubated for 20 days. Each reported result is the average of six closely agreeing determinations.

TABLE XI.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil, with and without arsenic, inoculated with soil extract*

Temperature of soil extract (°C.).	0.0728 gm. of lead arsenate added.	No arsenic added.
Room	8.77	5.11
50	9.24	9.00
55	14.28	14.14
60	12.60	16.38
65	13.85	14.42
70	12.18	13.02
75	12.88	11.34
80	13.44	12.66
85	11.54	10.36

The heating of the soil extract to a temperature of 55° C. for 15 minutes changes the soil so that it is no longer stimulated by arsenic. The heating of the soil extract to a higher temperature stimulates its nitrogen-

fixing properties. It is not, however, increased by the addition of arsenic. Hence, it would appear as if the substance which is suppressed by the arsenic is very thermolabile and is easily injured by drying, for it has been repeatedly brought to our attention that the drying of the soil prevents the arsenic from greatly stimulating its nitrogen-fixing properties. Harden and Young (1911, p. 72; 1906) have shown that the addition of arsenates to a yeast-juice sugar solution greatly accelerates the rate of fermentation of such a mixture. The close analogy existing between the chemical properties of phosphorus and arsenic led to the idea that possibly the arsenic replaced the phosphorus in the reaction characteristic of phosphorus, but they found that this is not the case, for while the arsenic has an optimum concentration, as has the phosphorus, there was no direct relationship between the amount of arsenate added and the extra amount of fermentation, the arsenic in this way acting more like a catalyzer than does the phosphorus. Furthermore it was shown that fermentation can not proceed in the absence of phosphorus, even though there be present either arsenates or arsenites. The arsenic acts mainly as a liberator of the phosphorus from the hexosephosphates and does not of itself enter into the vital reactions of the cell as does the phosphorus.

These facts make it likely that a similar action may be exerted by the arsenic upon the bacteria. For these reasons a series of experiments was arranged in which the phosphorus had been replaced by arsenic. These were carried on in the nitrogen-free quartz sand. To each 100 gm. of the sand there was added the quantity of carefully tested nutrient without phosphorus found in 100 c. c. of Ashby's solution. To one-half of them was added the phosphorus, while to the other half there was added 0.0728 gm. of lead arsenate. They were each inoculated with 1 c. c. of a soil extract and then incubated the regular length of time. The nitrogen determinations were made on them and sterile blanks with the following results: When incubated with complete Ashby's solution and 0.0728 gm. of lead arsenate, 100 gm. of sand fixed 11.62 mgm. of nitrogen. Similar samples without phosphorus but with arsenic fixed 0.03 mgm., while without phosphorus or arsenic there was fixed 0.01 mgm. of nitrogen. The results for the set with the complete nutritive media show that sufficient of the soil extract was taken to get the nitrogen-fixing organism, and the results without phosphorus show that there was not sufficient phosphorus in the 1 c. c. of soil extract to furnish phosphorus for the organisms. These results show conclusively that arsenic can not replace phosphorus in the vital activities of the nitrogen-fixing organisms of the soil, and establish for this set of organisms what Stoklasa (1897) has established for the higher phanerogams, Molisch (Lafar, 1911, p. 37) for algæ, Günther (1897) for the molds, and Harden and Young (1906) for the yeasts.

There is still the possibility that the arsenic liberates the phosphorus from its insoluble compounds in the soil and thus makes it more available to the micro-organisms. If this be the case, one would think that the addition of soluble phosphates to the soil investigated would increase its nitrogen-fixing powers. Experiments, however, did not bear out this assumption, for just as large a quantity of nitrogen was fixed in the absence of the soluble phosphate as in its presence. This was probably due to the fact that the soil under investigation was well supplied in the natural condition with soluble phosphorus. But that the arsenic did have an influence upon the solubility of the phosphorus of the soil was shown by the following experiment: 100-gm. portions of the soil were placed in covered tumblers. Of these, 24 received 0.0728 gm. of lead arsenate each, while the other 24 received none. The moisture was made up to 18 per cent and incubated for 20 days. At the end of this time the water-soluble phosphorus was determined in 12 of the treated and 12 of the untreated soils by extracting with 500 c. c. of distilled water and determining the phosphorus in the extract (Greaves, 1910). As an average of the 12 closely agreeing determinations of the soil treated with arsenic there was obtained 0.59 mgm. of water-soluble phosphorus, while the untreated soils yielded 0.52 mgm. This is a slightly greater quantity in the arsenic-treated soil than in the untreated, which is probably due to the fact that more of the phosphorus had been changed in the body of the soil organisms to nucleoproteins or phosphoproteins. That this is the correct interpretation is shown by the results obtained from the remaining samples. Twelve of these samples, six with and six without arsenic, were digested for six hours with 100 c. c. of 12 per cent hydrochloric acid and the phosphorus determined in the filtrate. The other samples were ignited and the phosphorus extracted by the 12 per cent hydrochloric acid determined. The average of the results thus obtained is given in the tabular form below:

SAMPLES NOT IGNITED:	
Soil with arsenic	105.6 mgm. of phosphorus.
Soil without arsenic	100.0 mgm. of phosphorus.
Excess of acid-soluble phosphorus in soil with arsenic	5.6 mgm. of phosphorus.
SAMPLES IGNITED:	
Soil with arsenic	107.7 mgm. of phosphorus.
Soil without arsenic	100.8 mgm. of phosphorus.
Excess of acid-soluble phosphorus in soil with arsenic	6.9 mgm. of phosphorus.

This would give by the Schmoeger method 2.10 mgm. of organic phosphorus in the arsenic-treated soil, while in the untreated soil there was

only 0.80 mgm. of organic phosphorus. This excess of organic phosphorus could not have come from the water-soluble phosphorus, as there was a difference of only 0.07 mgm. in the two soils; hence, it must be concluded that the arsenic increases the solubility of the phosphorus. This, however, may be due either to a direct interchange between the insoluble phosphorus of the soil and the arsenic or to its action upon bacteria, which causes them to become more active in growth and formation of various acids which act upon the insoluble phosphates of the soil, rendering them soluble.

GENERAL CONSIDERATIONS

The data reported prove conclusively that the arsenical compounds, with the single exception of Paris green, stimulate the nitrogen-fixing organisms of the soil and that this influence varies qualitatively but not quantitatively with the various soils. The results also bring out the fact that both the anion and the cation of the compounds have a marked influence upon the growth of the organisms. With some compounds both the anion and cation act as stimulants, while with others one stimulates and the other is markedly toxic. It is likely that little or no influence is exerted upon the nitrogen-gathering organisms by the sodium (Lipman and Sharp, 1912), and that the stimulating influence noted with dilute solutions and the toxic influence exerted with more concentrated solutions are due entirely to the arsenic. It is quite likely that the stimulating influence which Rivière and Bailhache (1913) have found sodium arsenate to have upon wheat and oats is an indirect effect which is exerted upon the bacterial flora of the soil and which in turn influences the yield of the various grains.

Both the anion and cation undoubtedly act as stimulants in the lead arsenate. Stoklasa (1913) has shown that lead when present in soil stimulates the growth of higher plants. This he (1911) ascribes to the catalytic action of these elements on the chlorophyll. The results herein reported, together with those previously published (Greaves, 1913a), indicate that it is due to the influence of the compounds upon the biological transformation of the nitrogen in the soil. The fact that the lead plays no small part in the stimulating influence is borne out by the work of Lipman and Burgess (1914), who found lead to stimulate nitrifying organisms.

Paris green is toxic to the nitrogen-fixing organisms in the lowest concentration tested. This is due to the copper and not to the arsenic, as it is well known that the copper ion is a strong poison to many of the lower plants. Brenchley (1914) found it to be toxic to higher plants when present in water to the extent of 1 part in 4,000,000,000. Although Russell (1912, p. 47) states that it is not as toxic in soil as in water, Darbishire and Russell (1905) found it to be toxic in soils, and they failed to get a stimulating influence with it. Montemartini (1911)

has noted a stimulation with copper sulphate when used in dilute solutions. This, however, may have been due to the anion and not to the cation, as sulphates do stimulate plants by their action on insoluble constituents of the soil (Greaves, 1910, p. 298). The same interpretation could be placed upon the results obtained by Lipman and Wilson (1913) and also those reported by Voelcker (1913), in which they noted a stimulation with copper salts. Clark and Gage (1906) have found that very dilute solutions of copper have an invigorating influence upon bacterial activity. In order that the stimulation may be noted the copper must be present in small quantities. Jackson (1905) found that 1 part of copper sulphate in 50,000 parts of water killed *Bacillus coli* and *B. typhosus*. Kellerman and Beckwith (1907) found that the common saprophytic bacteria are more resistant to copper than is *B. coli*. There is considerable evidence (Lipman and Burgess, 1914; Greaves, 1913a, p. 8) that copper stimulates the ammonifying and nitrifying organisms of the soil, but these results show the nitrogen-fixing organisms of the soil to be very sensitive to copper, and if it does act as a stimulant it must be in extremely dilute solutions. The toxicity of the copper in the Paris green is great enough in the dilution of 10 parts in 1,000,000 to offset the great stimulating influence of the arsenic in combination with it.

The very marked stimulating influence noted where the arsenic trisulphid is used is very probably due to both the arsenic and the sulphur. Demolon (1913) attributed much of the fertilizing action of sulphur to its action upon bacteria, and Vogel (1914) found that sulphur decidedly increased the activity of the nitrogen-fixing organisms. The results which Russell and Hutchinson (1913, p. 173) obtained with calcium sulphid are interesting in this connection. They found that after 30 days there were five times as many organisms in the soil to which calcium sulphid had been added as in the untreated soil, and the yield of ammonia and nitrates in this time was one-third greater in the treated soil than in the untreated soil. This, in turn, reacts upon the crop harvested, as shown by Shedd (1914, p. 595).

The first part of the curve (fig. 1) for the zinc arsenite nearly coincides with that of the sodium arsenate, but the zinc arsenite stimulates in greater concentrations than does the sodium arsenate. This is partly due to the difference in solubility of the two compounds, but there is another factor which enters, and that is that the zinc also acts as a stimulant. Latham (1909) found that small quantities of zinc stimulated algæ. The same results have been obtained by Silberberg (1909) in working with higher plants. Ehrenberg (1910) concludes that zinc salts are always toxic when the action is simply on the plant, but that they may lead to increased growth through some indirect action on the soil. He found that zinc stimulated plant growth in soils, but when the soil was sterilized the zinc became toxic. Lipman and Burgess (1914, p. 133)

have shown that it does stimulate the nitrifying organisms and that the influence is shown by the yield obtained from such soils (Lipman and Wilson, 1913). The great variation in the results reported by the various investigators for zinc, arsenic, and lead is probably due to the fact that it modifies the bacterial flora of the soil, and when heated soil or water cultures are used a different result is noted. This, however, is not the only factor which enters, for these results show a marked difference in soil and in water. The lead arsenate stimulates the nitrogen-fixing organisms when placed in soils but becomes very toxic to the same organisms when placed in nutritive solutions.

The difference is due in part to the adsorption of the soil, but in this case we would have to attribute it to the silica compounds of the soil, for the nitrogen-fixing organisms are stimulated by arsenic in quartz sand

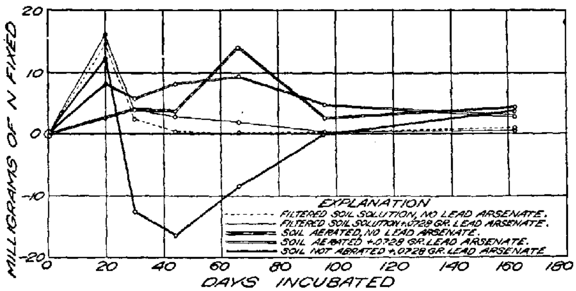

FIG. 2.—Graph showing the effect of aeration on the nitrogen-fixing activity of soil containing compounds of arsenic.

free from organic colloids. In this case the arsenic becomes concentrated at the surface, layers of the silica leaving the inner part of the water film comparatively free from arsenic, in which the micro-organisms multiply and carry on their metabolic processes. This being the case, one should, and probably could, find a water solution weak enough to stimulate bacteria. A great difference, however, between the solution and the sand-culture method is the greater aeration in the latter than in the former. That the aeration of a cultural medium does play a great part in determining the activity of the nitrogen-fixing powers of a soil is very strikingly brought out in figure 2. The graphs in this figure are made from the data given in Tables IV, V, and X.

It is remarkable how the aeration of the soil or the filtering of the soil extract can prevent the great loss of nitrogen which is noted at first in the unaerated soil. This can not be attributed directly to the denitrifying organisms; otherwise it would not be removed by filtration. The graphs

also bring out the fact that the addition of arsenic and the filtering of the soil only shift for the time the equilibrium within the soil, and later it tends to regain its old equilibrium. This is a condition which coincides well with what one would expect if the limiting element were some other microscopic forms of life. The filter would not separate them quantitatively, and it is possible that the arsenic has only a selective influence. Later, many of the organisms become accustomed to its presence; or, what is more likely, the arsenic becomes fixed (McGeorge, 1915) within the soil.

That this limiting factor is a thermolabile body is brought out more clearly in figure 3, which is made from the data reported in Table XI.

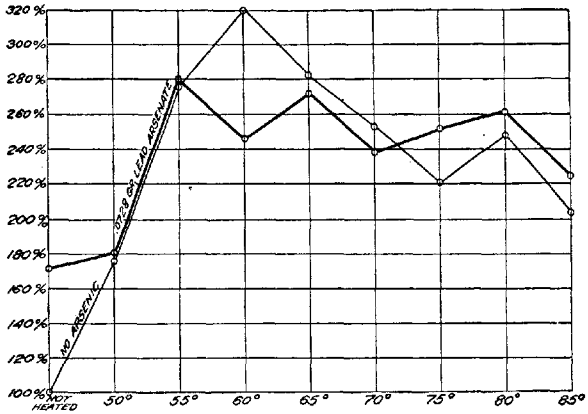

FIG. 3.—Graph showing the effect of heat on the nitrogen-fixing power of soil treated and not treated with arsenic.

The quantity of nitrogen fixed by the unheated soil receiving no arsenic has been taken as 100 per cent, and the heated soil with and without arsenic is compared with this.

The heating of the soil extract to 50° C. for 15 minutes has exactly the same influence measured in terms of nitrogen fixed as does 0.0728 gram of lead arsenate. The stimulating influence of heat is noted even in the presence of arsenic and reaches its maximum effect in the absence of arsenic at 60°, while in the presence of arsenic at 65° above these temperatures there is a decline in the nitrogen fixed. But even the soil inoculated with solutions which had been heated to a temperature of 85° fixed nitrogen; or at least there is more nitrogen accumulated in such soil than in that inoculated with the untreated soil solution. The results indicate that many of the organisms which take part in the gathering of nitro-

gen in this soil are very resistant to heat. It is also significant that the greatest stimulating influence is exerted in soil which had been inoculated with solutions heated just above what Cunningham and Löhnis (1914) found to be the thermal death point of soil protozoa.

The data presented in this paper, together with these presented in former publications, make it possible to compare the sensitiveness of the ammonifying, nitrifying, and nitrogen-fixing organisms toward the various arsenical compounds. Figure 4 represents the percentage of activity of the various classes of organisms in the presence of 400 p. p. m. of arsenic in the form of the various arsenical compounds. The untreated soil has been taken in every case as 100. The ammonifying organisms are retarded more by the lead arsenate than the nitrogen-fixing or nitrifying organisms. The latter two are influenced in nearly the same way by this concentration of lead arsenate. All three types of organisms are influenced in the same order by the arsenic trisulphid, while with the zinc arsenite the nitrogen-fixing and nitrifying organisms act about normally in concentrations of 400 p. p. m. of arsenic, but the ammonifiers are greatly depressed. Paris green stimulates the nitrifiers, but greatly depresses the other types of organisms. The results, with the exception of copper, show that the nitrifying and nitrogen-fixing organisms are very similar.

In figure 5 are shown graphically the quantities of arsenic in the form of various arsenicals which are required by the different organisms to give the greatest stimulation.

It has been shown that stimulation within a specific group of organisms varies with the quantity of water-soluble arsenic and the stimulating influence of the electropositive ion associated with the arsenic. But when we examine stimulation by these substances with different groups of organisms, we find a marked difference which can not be attributed to solubility but must be due to a physiological difference existing in the various organisms; for instance, the nitrogen-fixing organisms require 200 p. p. m. of arsenic in the form of lead arsenate for the greatest stimulation, while the nitrifiers and ammonifiers require much smaller quantities. For maximum stimulation with arsenic trisulphid the nitrogen-fixing organisms require the greatest concentration, folowed by the nitrifying and ammonifying organisms in the order given. Zinc arsenite, on the other hand, has to be present in large quantities for a maximum stimulation of the nitrifying organisms, while very small quantities give a maximum stimulation with the other two groups of organisms. Practically the same order is followed by the organisms in the presence of sodium arsenate and Paris green, there being, however, this significant difference, that neither the ammonifiers nor the nitrogen-fixing organisms are stimulated in any concentration by the presence of copper, and it is quite possible that the same holds for the nitrifying

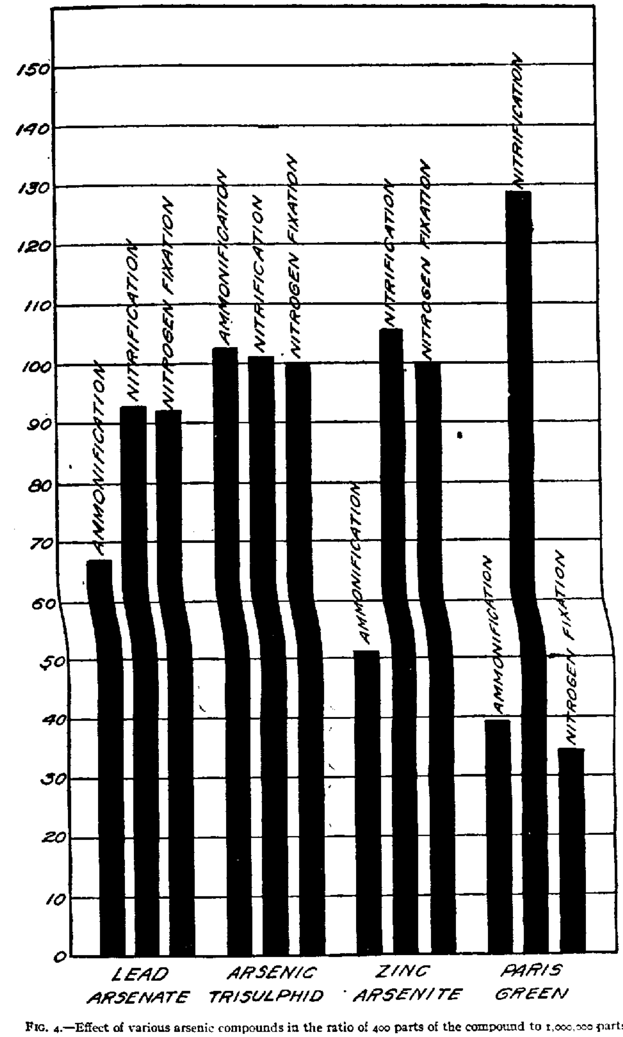

FIG. 4.—Effect of various arsenic compounds in the ratio of 400 parts of the compound to 1,000,000 parts of soil on the activity of various soil organisms.

organism. This set of organisms are, however, more resistant to copper than are others, and what we have occurring is a suppression of other types which feed on nitrates, thus permitting a greater accumulation

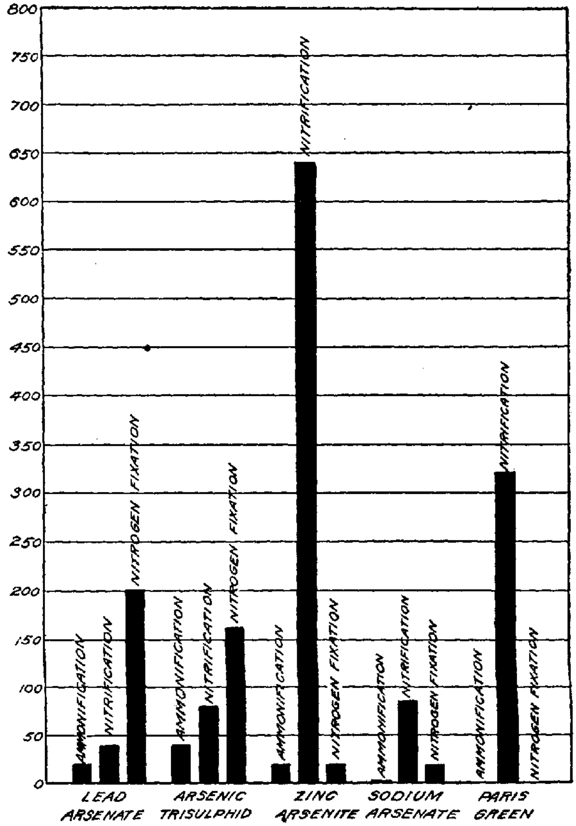

FIG. 5.—Graph showing parts per million of various arsenic compounds in the soil at which the greatest stimulation occurred.

of nitrates under these conditions. While not so likely in the other cases, the same possibility does arise. This, however, can be answered definitely only by further experiments.

SUMMARY

Arsenic, when applied to a soil in the form of lead arsenate, sodium arsenate, arsenic trisulphid, or zinc arsenite, stimulates the nitrogen-fixing powers of the soil. This stimulation is greatest when lead arsenate is applied and least when zinc arsenite is applied. Paris green did not stimulate in any of the concentrations. This compound becomes very toxic when the concentration reaches 120 p. p. m. The toxicity of this compound is due to the copper and not to the arsenic contained in it. Sodium arsenate became toxic when a concentration of 40 p. p. m. of arsenic was added, and when 250 p. p. m. were added it entirely stopped nitrogen fixation. Lead arsenate was not toxic even at a concentration of 400 p. p. m. of arsenic. The toxicity of arsenic trisulphid and zinc arsenite was very small at this concentration.

The stimulation noted when arsenic is added to a soil is not due to any inherent peculiarity of the soil used, for soils which vary greatly in physical and chemical properties had their nitrogen-fixing powers greatly increased when arsenic was applied to them. Soils high in organic matter fixed as much nitrogen in the presence of arsenic and in the absence of mannite as they did in the presence of mannite and absence of arsenic. The stimulation is greatest when the water-soluble arsenic content of the soil is about 10 p. p. m. This quantity exceeds that found in most soils, so it is likely that in agricultural practice arsenic will stimulate and not retard bacterial activity in the soil.

Only one type of Azotobacter was isolated which was stimulated by arsenic, and in this case the stimulation was due to the organism utilizing more economically in the presence of arsenic its source of carbon than it did in the absence of arsenic. The arsenic compounds do not act as a source of energy to the organisms. The main part of the stimulation noted in the soil with its mixed flora is undoubtedly due to the arsenic inhibiting injurious species.

A quantity of arsenic which acts as a stimulant to bacteria when placed in soil may become very toxic when tested by the Remy-solution method.

Arsenic can not replace phosphorus in the vital process of the nitrogen-fixing organisms, but it can in some manner liberate the phosphorus from its insoluble compounds. This may be either a direct or an indirect action.

Arsenic stimulates the cellulose ferments, and these in turn react upon the activity of the nitrogen-fixing organisms.

The nitrogen-fixing powers of soil extract, of filtered soil extract, and soil dried for some time are only slightly stimulated by arsenic, showing that arsenic acts mainly by the removal of a thermolabile body which occurs in the soil.

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TRANSMISSION AND CONTROL OF BACTERIAL WILT OF CUCURBITS [1]

By FREDERICK V. RAND, *Assistant Pathologist*, and ELLA M. A. ENLOWS, *Scientific Assistant, Laboratory of Plant Pathology, Bureau of Plant Industry*

WILT TRANSMISSION

That the striped cucumber beetle (*Diabrotica vittata* Fab.) is a direct carrier of the bacterial-wilt organism (*Bacillus tracheiphilus*) from infected to healthy cucurbits was shown several years ago by Smith.[2] He also expressed the conviction that it was the most important, if not the only, summer carrier, and stated the possibility of its serving also as the winter carrier of the disease. Observation and experiment by the senior writer [3] during the last two seasons have abundantly confirmed the implication of the striped cucumber beetle as a summer carrier and have brought out strong proof that this insect is not only the principal summer carrier but also the winter carrier of the wilt organism. The twelve-spotted cucumber beetle (*D. duodecimpunctata* L.) must be included with the striped cucumber beetle at least as an important summer carrier of the disease.

INSECT TRANSMISSION

Relative to cucumber beetles as winter carriers, several direct cold-storage tests have been carried out by the writers in Washington. During the summer and fall of 1915 hundreds of beetles were collected and placed in cold storage at temperatures ranging from 4° to 10° C. These early experiments were conducted partly with a view to determining the proper conditions of feeding prior to storage and the temperature and humidity most favorable to hibernation in storage. The optimum environment for hibernation varies for different insects, and it is necessary to work out this problem for each species. Consequently in these preliminary tests the greater portion of the beetles placed in cold storage was lost. Infection experiments with the few surviving beetles gave the results here detailed.

EXPERIMENT 1.—Several striped cucumber beetles were collected in October, 1914, and fed about two weeks on cucumber vines (*Cucumis sativus*) wilting as a result of natural infection with *B. tracheiphilus*. After six weeks' hibernation in cold storage the five surviving beetles were caged with a young squash plant on which

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[1] Some of the details of the field experiments at East Marion, N. Y., were carried out by Mr. Wayland C. Brown, of the Bureau of Plant Industry. The land used in these experiments was furnished by Messrs. J. H. Douglass and G. S. Nowell, of East Marion.

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[3] Rand, F. V. Dissemination of bacterial wilt of cucurbits. *In* Jour. Agr. Research, v. 5, no. 6, p. 257-260, pl. 24. 1915.

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they were allowed to feed for 11 days. Observation after two weeks showed unmistakable signs of incipient wilt around some of the beetle injuries on the leaves— that is, a lighter dull green and slight flaccidity of the tissues. With the expectation that the wilt would extend throughout the leaves the pouring of plates was deferred. However, these incipient infection areas dried up without spreading further, and consequently it was impossible to obtain cultures. That *B. tracheiphilus* was present in the wilted vines fed to these beetles was shown by the subsequent inoculation of cucumbers, cantaloupes, and squashes with cultures obtained from these wilted vines (strains R230 and R235). Numerous inoculations with these two strains have shown them to be virulent upon cucumbers and cantaloupes, but inoculations on several varieties of squash have given nothing more than incipient infection.

EXPERIMENT 2.—On October 25, 1915, striped cucumber beetles were collected at Giesboro Point, D. C., in a squash field where bacterial wilt was very prevalent. These beetles were fed for three days on plants which were wilting as a result of inoculation with pure cultures of *B. tracheiphilus*. They were then placed in small boxes provided with screened covers, and held in the ice compartment of a refrigerator at a temperature of about 10° C. for five weeks and four days. At the end of this time (Dec. 6) the beetles were removed and placed in cages containing young cucumber plants. Four to six beetles were placed in each of the six cages used, each cage containing three young plants. After being allowed to feed on these plants for 10 days the beetles were removed and the plants kept in one of the Department greenhouses where there had been no cucurbit wilt since the preceding spring and where no cucurbitaceous insects were present.

On December 17 leaves injured by the beetles on three of these plants were wilted. Microscopic examination showed bacteria present in great number in the vessels of the petioles, and poured plates from the wilted leaves and petioles gave pure cultures of the wilt organism (strain R313). Needle-prick inoculations from these cultures again gave typical wilt on cucumber plants. On December 24 a gnawed leaf on a fourth plant was found wilting, and was removed from the plant. Enormous numbers of bacteria were present in the vascular tissues, and cultures (strains R315 and En126) isolated therefrom gave also successful infection when pricked into the leaves of young cucumber plants. From the portion of petiole remaining the wilt gradually extended throughout the plant, which finally collapsed. On January 4 another plant was found entirely wilted. The gnawed leaf which had wilted first, and from which the wilt had spread throughout the plant, was photographed and preserved. Cultures (strain En124) and paraffin sections (En36) were made from the petiole of this leaf. The organism isolated gave typical infections when inoculated into cucumber plants.

EXPERIMENT 3.—Another lot of *D. vittata* collected in the squash field referred to in experiment 2 was fed for three days on old wilting stems of squash (*C. maxima*) collected in the same field. After keeping these beetles in storage for two months under the same conditions as in experiment 2, they were removed and caged for five days with 12 young cucumber plants. Although these plants were under observation for over two months no wilt appeared in any of them.

EXPERIMENT 4.—On December 16, 1915, five specimens of *D. vittata* and four of *D. duodecimpunctata* hibernating under natural conditions in the squash field at Giesboro Point, D. C., were sifted from the surface soil and taken to the greenhouse. The striped and spotted beetles were placed at once in separate cages, each containing three young cucumber plants. Although the beetles fed freely on these plants, the results of this experiment were negative.

The negative results in experiment 3 possibly may be explained by the fact that the wilted plants fed to the beetles were old, ripe squash

vines which had been diseased for a long time. Doubtless few living organisms were present, since great difficulty was experienced in obtaining cultures of *B. tracheiphilus* from this field (strains En102 and En110). The beetles used in experiment 4 were collected when hibernating in a field where wilt was known to have occurred, but it is evidently not possible to determine whether they had fed upon wilted plants. On the other hand, it is not reasonable to assume that all beetles which have fed upon wilted plants would necessarily be able to carry infection on their mouth parts for any great length of time. Experiments 1 and 2 show that at least in some cases the striped beetles may carry the wilt organism for at least five or six weeks and still be able to infect healthy plants. This, in connection with the field experiments previously published,[1] seems to establish beyond doubt that *D. vittata* is a winter carrier of the cucurbit organism.[2] Experiments with other species of insects have thus far given negative results, as here detailed.

In each of seven tests carried out with the common squash bug (*Anasa tristis* DeG.) during the summer and fall of 1915 in field and greenhouse, two to six of these insects were fed for one to three days on wilted cucumber leaves and petioles and then inclosed with several healthy cucumber plants. After feeding on these plants for one to two days the bugs were removed and the plants kept under observation for three to four weeks. No wilt appeared in any of these plants, but no absolute conclusion can be drawn from the negative results of so small a series of tests.

The twelve-spotted (or squash) lady beetle (*Epilachna borealis* Fab.) was very scarce in eastern Long Island during the season of 1915, but two tests with it similar to those outlined above gave negative results.

The melon aphis (*Aphis gossypii* Glov.) and the flea beetle (*Crepidodera cucumeris*) apparently do not serve as wilt carriers. This has been shown by the negative results from transfer of insects fed upon wilted plants to healthy cucumber plants in insect-proof cages (three tests), and by the fact that no wilt developed during the season in cucumber plants grown in 48 large screened cages (East Marion, Long Island, N. Y., 1915), although numerous wilted plants occurred around all of these cages, and aphids and flea beetles had free access through the meshes of wire netting and were abundant both outside and inside the cages.

In only 2 out of 50 cages did wilt appear and in these cases striped cucumber beetles had gained access or had been purposely introduced, and the disease had started from points gnawed by the beetles.

[1] Rand, F. V. Op. cit.

[2] Wild cucurbits may be eliminated as possible carriers of bacterial wilt so far as the experiments at East Marion are concerned. Personal observations, together with those of Burnham and Latham (Burnham, Stewart H., and Latham, Roy A. The flora of the town of Southold, Long Island and Gardiner's Island. *In* Torreya, vol. 14, nos. 11–12, 1914), and a search through the herbaria of the New York and Brooklyn Botanical Gardens, have established beyond doubt that no wild Cucurbitaceae occur within 10 to 15 miles of the experimental plots.

In each of eight direct summer field tests, one to five striped cucumber beetles were fed for one to three days on wilting cucumber leaves and petioles and then at once caged up with several healthy young cucumber plants. In six out of these eight tests bacterial wilt appeared in one to two weeks and only on plants gnawed by the beetles.

In the two fields (East Marion, Long Island, N. Y.) where spray tests were carried out during the season of 1915 the prevalence of bacterial wilt closely followed that of the striped cucumber beetle. Throughout the season careful and frequent observation failed to disclose a single case of wilt which had not evidently started in a part of the plant injured by cucumber beetles (Pl. LIII). In these two fields no wilt had appeared up to the 1st of July. A few cases were observed on July 3, while the greatest number of cases was found during the last 10 days of the month. Practically no new cases of wilt appeared after the 30th of July. The first striped cucumber beetles of the season were seen from June 15 to 17. In field 1 the first beetles were found on June 17 between cages 14 and 15.[1] On July 3 there were only seven cases of wilt in the whole field, and six of these occurred near or about where these beetles had been collected. The beetles were most numerous between June 24 and July 8, in fact so numerous that in order to save the plants from entire destruction an application of a proprietary dust insecticide (containing lime, Paris green, etc.) was made upon the unsprayed plots. Thus, for a few days, or until new growth appeared on the vines, there were no untreated cucumber plants in these two fields upon which the beetles could feed. From this date on, the beetles began to disappear from these fields. In the variety-test block and commercial fields in the vicinity the plants were younger and for the most part were untreated. In fact, most commercial plantings were just breaking through the ground on July 10. Such fields present an attractive feeding ground for the beetles. In the two experimental fields there were only a few beetles present on July 15, and they were exceedingly scarce after July 30.

When it is remembered that under field conditions usually one to three weeks elapse between time of infection and the appearance of wilting in the plants, it will be seen that the rise and fall in the number of plants with bacterial wilt closely follows the rise and fall in the number of beetles (fig. 1).

The two fields just discussed had been planted to cucumbers the preceding season. About a quarter of a mile from field 1 a cucurbit variety test block was located. This land had not been plowed for several years. Although separated only by slightly rolling, plowed land from field 1, where striped cucumber beetles appeared on June 17, no beetles appeared here until about the end of the first week in July. This was just after

[1] These beetles were used in the cage transmission tests recorded in a former paper (Rand, F. V., op. cit.) and mentioned in a preceding paragraph.

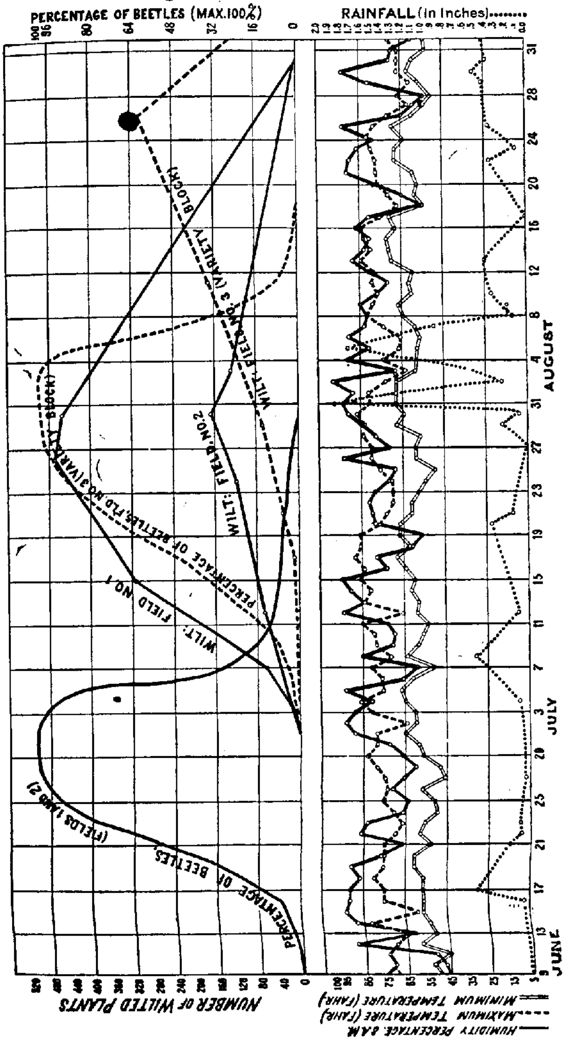

FIG. 1.—Comparison of the amount of wilt with striped-beetle prevalence and with meteorological phenomena in three fields, East Marion, Long Island, N. Y., season of 1915.

they had begun to disappear from field 1. In the variety test the first scattered cases of wilt were observed on July 17, whereas in field 1 the first cases were noted on July 3. The largest number of cases of wilt in the variety block were found between August 15 and 30, and the striped cucumber beetles were most numerous here during the last part of July. Again, allowing for the necessary time between infection and actual wilting, it will be noted that here also there is a direct relation between the number of wilt infections and the number of beetles present (fig. 1).

The graphs (fig. 1) show the daily relation between meteorological conditions, the number of beetles present, and the number of wilted plants in the three fields from June 10 to August 31. In these graphs there is shown a definite relation between the beetle and the wilt curves, but no relation between the latter and the meteorological curves. The meteorological instruments from which the data were obtained for this graph were kept in a United States Weather Bureau instrument shelter at ground level, so that the environment would correspond as nearly as possible to that of the cucumber plants (Pl. LIV, fig. 4).

Reference should be made to the fact that in taking notes the total number of plants showing bacterial wilt was recorded at each date of observation. This number included not only the new cases but also cases holding over from the preceding observation. Ordinarily the older the plant at the time of infection the longer the interval between infection and death. This explains the apparently too great interval between the maxima of the beetle and wilt curves. If it had been the original intention to represent graphically the relation between the prevalence of the beetles and the occurrence of wilt, the data would have been obtained in a form better suited to this method. It was only after tabulating the results of the field observations that the very striking parallel was noted. Obviously it would be impossible to enumerate absolutely the beetles present in a field; hence, the percentages used in the graphs are based partly on actual counts and partly on careful estimates made throughout the season. In the curves, 100 per cent represents the maximum number of striped cucumber beetles present at any one time.

Attention should be drawn to the fact that although there was a difference of only three days in planting time between field 1 and field 3, the beetles appeared between two and three weeks earlier in field 1, which had been planted to cucumbers the preceding season. This would suggest that these insects hibernated in or near the old cucumber field and that they did not leave this field the following spring as long as young and tender plants remained for them to feed upon. A similar tendency of both striped and twelve-spotted cucumber beetles to hibernate in old cucurbit fields was observed by the writers near Congress Heights, D. C. The first frosts occurred in these fields during the first part of October. About the middle of December, 1915, soil siftings to